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ICOS activation in combination with electrochemotherapy generates effective anti-cancer immunological responses in murine models of primary, secondary and metastatic disease

Liam F. Tremble [1], Morgan A. O'Brien [1], Patrick F. Forde[*, 2], Declan M. Soden [2]

Cork Cancer Research Centre, Western Gateway Building, University College Cork, Western Road, Cork, Ireland

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ABSTRACT

Electrochemotherapy is an evolving therapy which has recently been shown to induce an immunogenic form of cell death. It is hypothesized that the immunogenic cell death induced by electrochemotherapy may compliment the responses seen with anti-cancer immunotherapies. We therefore examined the effect of electrochemotherapy in combination with ICOS activation, which promotes the activity of previously activated T cells. In comparison to either monotherapy which resulted in no curative outcomes in any model, in a CT26 primary tumour 50% of mice were cured, with 100% of cured mice surviving tumour rechallenge. In a dual flank CT26 model mimicking secondary disease 20% of mice were cured, and 30% of mice were cured using an aggressively metastatic Lewis Lung Carcinoma model. We have shown the novel combination of electrochemotherapy with ICOS activation can inhibit local and distal tumour growth, including total tumour clearance with long lasting immunological memory.

1. Introduction

Tremendous progress has been made over the last decade in the development of immunotherapies for cancer patients. A range of novel treatment modalities have successfully improved survival, however low response rates and the development of therapeutic resistance are driving research to improve immunotherapy [10,19].

ECT was traditionally delivered to cutaneous tumours, as the devices delivering electrical pulses require direct tumour contact. The development of novel devices has however opened up access to deep seeded tumours.

Endoscopic devices have increased the range of cancers which can be treated by ECT to include cancers such as oesophageal and colorectal cancer. ECT has also shown to be a viable option in other tumours which require invasive procedures such as for the treatment of liver metastasis, and the translation to other tumour types is in progress [4].

Unlike cutaneous tumours which can be treated multiple times with relative ease, the medical requirements associated with accessing deep seeded tumours decrease the tolerability of the procedure for patients and places restrictions on the number of procedures which can be performed. As such, a major research goal of treatment with ECT is to develop effective treatment modalities which can be delivered in a single procedure.

As indicated by the explosion in immunotherapeutics, the design of effective anti-cancer therapeutics involves treatments which result in both a marked reduction in tumour volume and a systemic anti-cancer immune response resulting in the generation of immunological memory. Many local ablative modalities induce cell death which can provoke an immunological response, however this is not adequate to generate a sustained or effective anti-cancer response in the majority of cases. Utilizing the immunogenic potential of these local therapies to act as an adjuvant to other therapies which rely on the activation of the immune system may result in enhanced patient responses.

ECT is an evolving therapy which has shown the ability to induce an immunogenic form of cell death, cytotoxic T cell responses against the tumour type treated and immunological memory [2,11].

There have been a range of successful combinations of local treatments with immunotherapies providing proof of concept progressing through preclinical and clinical stages. Positive results have been seen in the combination of ECT with other

* Corresponding author.
E-mail address: p.forde@ucc.ie (P.F. Forde).
[1] These authors have contributed equally and are joint first author.
[2] These authors have contributed equally and are joint senior author.

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Abbreviations

CRS	Cytokine release syndrome
DMEM	Dulbecco's Modified Eagle Medium
ECT	Electrochemotherapy
FBS	Foetal bovine serum
ICOS	Inducible T-cell costimulator
LLC	Lewis Lung Carcinoma
PBS	Phosphate buffered saline
SEM	Standard error of the mean

immunotherapies. IL-12 is a proinflammatory cytokine which promotes the polarization of naïve T cells towards a Th1 type phenotype. Combination of ECT and electro-gene-transfer of plasmids encoding IL-12 have shown enhanced survival, with the induction of innate and adaptive immune responses characterized by infiltration of T cells and macrophages [9,18,20].

Local ablative techniques, including ECT, in combination with Ipilimumab have been shown to significantly prolong overall survival in advance melanoma, regardless of BRAF mutational status [23,24]. Further clinical trials are ongoing examining sequential therapy of ECT followed by Ipilimumab in patients with advanced melanoma [15].

Inducible T-cell costimulator (ICOS) is a member of the CD28 family of receptors. Unlike other drugs which focus on blocking inhibitory pathways on T cells, ICOS is a receptor which delivers stimulatory signals to T cells [3]. Activating anti-ICOS antibodies are currently in development for a range of solid tumours, alone and in combination with other T cell checkpoint inhibitors, such as GSK3359609 (GlaxoSmithKline) and JTX-2011 (Jounce Therapeutics) which have progressed to phase I/II clinical trials [6]. The restriction of ICOS expression to T cells which have already recognized antigen results in the expansion and activation of a range of anti-tumour T cell clones [8,26,27].

Here we examine the combination of an activating ICOS antibody with electrochemotherapy (ECT). ECT is a local treatment modality that results in an immunogenic form of cell death [2]. During this process a range of damage associated molecular patterns (DAMPs) are released locally and induce a proinflammatory immune response. We hypothesized that the immunogenic cell death generated by ECT would be synergistic with a T cell activating ICOS antibody. Here we report improved efficacy of an ICOS agonistic antibody by the addition of ECT in murine models of colorectal cancer, secondary disease, and metastasis, resulting in decreased tumour volume, enhanced overall survival and the generation of long lasting immunological memory.

2. Materials and methods

2.1. Cell culturing

Lewis Lung Carcinoma (LLC) and CT26 cells were obtained from the European Collection of Authenticated Cell Cultures and both were maintained in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% foetal bovine serum (FBS), 2 mM L-glutamine, 50 I·U/mL penicillin and 50 μg/mL streptomycin.

2.2. Ethics statement

All animal husbandry and experimental procedures were approved by the University College Cork Animal Experimentation Ethics Committee and carried out under licenses issued by the Department of Health, Ireland as directed by the Cruelty to Animals Act Ireland and EU Statutory Instructions.

2.3. Tumour induction & monitoring

For tumour induction, 1×10^6 CT26 cells or 2×10^5 LLC cells in a 200 μL volume of serum free DMEM were injected subcutaneously into the right flank of 6–8 week old BALB/C or C57BL/6 mice respectively. For dual flank tumour induction the left flank was injected with the same number of CT26 cells 4 days later. To determine the metastatic burden, mice were culled 7 days post the commencement of treatment, intact lungs were harvested and weighed. Tumour volume was measured using a Vernier callipers and the formula $ab^2\pi/6$ was used to calculate the tumour volume where a is the greatest cross sectional distance and b is the greatest width perpendicular to a. Day zero in all graphs represents the day in which treatment commenced.

2.4. Tumour rechallenge

Tumour free, 'cured' mice from the original CT26 single flank study were rechallenged subcutaneously, on the contra-lateral flank, with the same concentration of CT26 cells (1×10^6 cells per 200 μL in serum free media) 90 days after total regression of the initial tumours on right flank. 6–8 week old naïve mice also had CT26 tumours induced on the same flank, using the same cell concentration, at the same time.

2.5. Electrochemotherapy

Tumours were treated once they reached an approximate size of 0.4 cm × 0.4 cm approximately 12–14 days after tumour induction. 1 mg/kg cisplatin was made up to a volume of 200 μL and injected intratumourally in a fan pattern 10mins prior to electroporation. Electrical parameters applied to the tumour were for needle electrodes type II, linear (C) with 8 pulses of 1300 V/cm and 100μs duration, delivered at 1 Hz using a Cliniporator™ device (IGEA S.R.L, Carpi, Italy) and a unidirectional arrangement of needle electrodes with 0.4 cm inter-needle distance. The process was repeated as many times as necessary to cover the entire surface area of the tumour including all margins of at least 0.2 cm and covering the tumour in a circular fashion starting at the perimeter and working towards the centre of the tumour.

2.6. ICOS stimulation

The anti-ICOS agonist antibody (clone C398.4 A, Biolegend) was made up to required concentration in sterile PBS and administered intraperitoneally at a dose of 100 μg/100μL per mouse. Animals were dosed within 12 hrs post ECT treatment and subsequently every 12 hrs for a total of 3 doses.

2.7. Processing tumour tissue for flow cytometry analysis

LLC tumours were processed into fine pieces using a sterile scalpel, and incubated in 3 mg/ml of collagenase and dispase at 37 °C for 10 min. The sample was mixed and passed through two 70 μm nylon cell strainers. The cells were washed twice in PBS and resuspended in 2 ml of red blood cell lysis buffer (Sigma) for 5 mins at room temperature. Lysis was stopped by adding 30 ml DMEM media with serum. For flow cytometry staining a buffer of PBS with 1% bovine serum albumin, 0.1% sodium azide, 1% mouse serum and 3% buffered formalin was used. Cells were stained using CD8, CD19, CD11c (all BD biosciences), CD44, CD183, CD9 (all eBioscience) and